

Mouse CD8⁺ T Cell Isolation Kit (positive isolation)

Catalog No.: BRC0005

Product Description

The Mouse CD8⁺ T Cell Isolation Kit (positive selection) is designed to isolate CD8⁺ T cells from mouse splenocytes or single-cell suspensions of other tissues. The principle utilizes CD8 Capture Antibody to label CD8⁺ cells, followed by capture with Releasable EnkiBeads™. The EnkiBeads™ Release Buffer is then used to dissociate the magnetic beads from the cell surface, yielding bead-free mouse CD8⁺ cells. The isolated CD8⁺ cells can be used for downstream molecular and cellular biology applications.

Kit Components

No.	Reagent Name	Size1 (For 10 ⁹ cell)
Reagent1	CD8 Capture Antibody	200μl
Reagent2	Releasable EnkiBeads™	2 mL
Reagent3	EnkiBeads™ Release Buffer	40mL

Storage Conditions and Shelf Life

Storage Conditions: Store at 2–8°C. DO NOT FREEZE.

Shelf Life: 2 years

Applications

This kit is suitable for isolating CD8⁺ T cells from mouse spleen and lymph nodes.

Protocol

Example: Isolation of CD8⁺ T Cells from Mouse Spleen

1. Prepare single-cell suspension: Mince spleen through a 70 μm cell strainer. Rinse the strainer with ice-cold PBS and collect the cell suspension in a 50 mL centrifuge tube. Centrifuge at 500 g for 5 min.
2. Remove supernatant after centrifugation, add 5 mL erythrocyte lysis buffer (Cat. No. RC0011), and lyse at room temperature for 5 min. Add 20 mL PBS and centrifuge at 500 g for 5 min.

Note: Erythrocyte lysis conditions may vary depending on the specific lysis buffer used. Trace residual erythrocytes will not affect subsequent isolation or cell purity.

3. After centrifugation, remove supernatant, resuspend splenocytes in PBS, filter through a 70 μm cell strainer, and count cells. Centrifuge at 500 g for 5 min.

Note: The cell suspension must be filtered through a cell strainer to remove tissue debris and cell aggregates; otherwise, isolation purity will be compromised.

4. After centrifugation, remove supernatant and resuspend cells in isolation buffer at a density of 1×10⁸ cells/mL.

Note: Isolation buffer is PBS containing 2 mM EDTA and 2% fetal bovine serum (FBS), or PBS containing 2 mM EDTA and 0.5% BSA. Filter-sterilize through a 0.22 μm membrane before use.

5. Transfer 500 μL of cell suspension (5×10^7 cells) to the bottom of a sterile flow cytometry tube, add 10 μL CD8 Capture Antibody, mix well, and incubate at 4°C for 15 min.

Note: When adding cell suspension, pipette directly to the bottom of the tube; avoid dispensing along the tube wall. Depending on the magnetic separator used, centrifuge tubes may also be used for cell isolation. For different cell numbers, adjust CD8 Capture Antibody proportionally. For fewer than 1×10^7 cells, adjust cell suspension volume to 100 μL and add 2 μL CD8 Capture Antibody.

6. After incubation, add 100 μL washed Releasable EnkiBeads™ to the tube (magnetic beads must be washed before use: vortex to resuspend beads, pipette the required volume into a 1.5 mL centrifuge tube, add 1 mL isolation buffer, centrifuge at 10,000 g for 1 min, and remove supernatant. Repeat wash once with 1 mL isolation buffer, then resuspend beads in the same original volume of isolation buffer. For example, if 20 μL of beads were taken for washing, resuspend in 20 μL isolation buffer after washing), mix well, and incubate at 4°C for 10 min.

Note: For different cell numbers, adjust Releasable EnkiBeads™ proportionally. For fewer than 1×10^7 cells, use 20 μL Releasable EnkiBeads™.

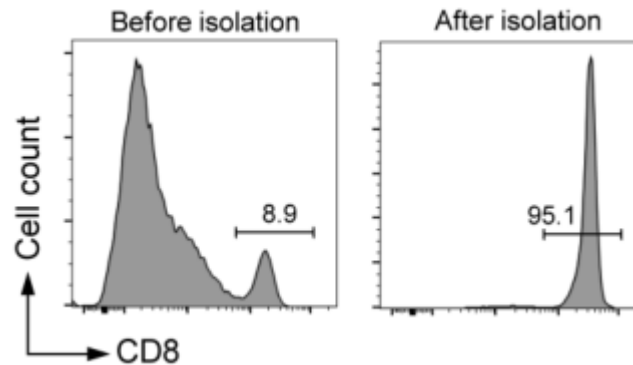
7. After incubation, add isolation buffer to the tube to a final volume of 2.5 mL, mix by pipetting up and down 5 times (avoid inversion mixing). Place the tube on the magnetic separator and let stand for 5 min.
8. Aspirate and discard the supernatant. Remove the tube from the magnetic separator, quickly add 2 mL isolation buffer, and resuspend beads by repeated pipetting. Place the tube on the magnetic separator and let stand for 5 min.
9. Repeat step 8 twice (thorough washing ensures subsequent elution of high-purity target cells).
10. After magnetic separation, aspirate and discard the supernatant. Remove the tube from the magnetic separator, quickly add 1 mL EnkiBeads™ Release Buffer to resuspend beads (avoid bead drying), transfer the bead suspension to a 1.5 mL centrifuge tube, and incubate with rotation at room temperature for 10 min.

Note: For different cell numbers, adjust EnkiBeads™ Release Buffer proportionally. For fewer than 1×10^7 cells, use 200 μL EnkiBeads™ Release Buffer to elute cells.

11. After incubation, pipette up and down at least 10 times, transfer the bead suspension to a new flow cytometry tube, add isolation buffer to 2.5 mL, mix by pipetting, and place the tube on the magnetic separator for 5 min.
12. Transfer the supernatant to a 15 mL centrifuge tube and set aside (the supernatant contains target cells, do not discard). Quickly resuspend beads in 1 mL EnkiBeads™ Release Buffer (avoid bead drying), transfer the bead suspension to a 1.5 mL centrifuge tube, and incubate with rotation at room temperature for 10 min.
13. After incubation, pipette up and down at least 10 times, transfer the bead suspension to a new flow cytometry tube, add isolation buffer to 2.5 mL, mix by pipetting, and place the tube on the magnetic separator for 5 min.
14. Pool the supernatant with the cell supernatant from the first elution, centrifuge at 500 g for 5 min, and remove supernatant to collect bead-free CD8⁺ cells.
15. Wash cells as required for your experiment, then resuspend in appropriate buffer or culture medium for subsequent molecular or cellular biology applications.

Isolation Performance

CD8⁺ cells were isolated from BALB/c mouse splenocytes and stained with PE-conjugated anti-mouse CD8 antibody (clone 53-5.8) for flow cytometry analysis. CD8⁺ cell purity increased from 8.9% to 95.1% pre- and post-isolation.



Important Notes

1. Avoid freezing magnetic beads and antibody mix during storage and use.
2. Use low-retention pipette tips and centrifuge tubes to minimize loss of beads and antibodies due to adsorption.
3. This product should be used with a magnetic separator.
4. For research use only.